Date: 20170928
Operators: Ersin, Gaétan, Deshmukh

Miniprep on pEX-S1 transformed Bacteria

Transformed Bacteria
- DH5α transformed with pEX-S1

Miniprep :

Aim: retrieve amplified plasmids from colony of transformed bacteria
QIAGEN kit: QIAprep Spin Miniprep Kit

1. Pellet 1-5 ml bacterial overnight culture by centrifugation at 3 500 x g for 3 minutes at room temperature (15-20°C) (Done by Deshmukh the 27th of September).
2. Resuspend pelleted bacterial cells in 250 µl buffer P1 and transfer to a microcentrifuge tube (1.5 Eppendorf tubes)
3. Add 250 µl buffer P2 and mix thoroughly by inverting the tube 4-6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5 minutes.
4. Add 350 µl buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times.
5. Centrifuge for 10 minutes at 16 100 x g in a table-top microcentrifuge.
6. Apply the 800 µl supernatant from step 5 to the QIAprep 2.0 spin column by pipetting. Centrifuge for 60 seconds at 16 100 x g and discard flow-through.
7. Wash the QIAprep 2.0 spin column by adding 0.75 ml buffer PE. Centrifuge for 60 seconds at 16 100 x g and discard the flow-through. Place the QIAprep 2.0 spin column back in the collection tube.
8. Centrifuge for 1 minute at 16 100 x g to remove residual wash buffer.
9. Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 25 µl Buffer EB (Tris-Cl 10 mM, pH 8.5) to the center of the of the QIAprep 2.0 spin column, let stand for 5 minutes, and centrifuge for 1 minute at 16 100 x g.
10. Repeat step 9 with another 25 µl of buffer EB (Tris-Cl 10mM, pH 8.5) to the center of the of the QIAprep 2.0 spin column, let stand for 5 minutes, and centrifuge for 1 minute at 16 100 x g.
11. Measure the DNA concentration using the UV5 spectrophotometer machine. 3 measurement of 3 µl each. Cf UV5 protocol.

Measure the DNA concentrations using the UV5 machine.

UV5 spectrophotometer use for concentration analysis:

1. Buffer: water or $TE_{0.1}$ 1X
2. Start the program iGEM_DNA (2 µl)
3. Clean the lens with sterile water on a tissue
4. Add **3 µl** of solution Buffer on the lens (no bubbles, no overflow of the solution outside the lens)

5. Analyse the solution
6. Clean the lens with a tissue and sterile water
7. Vortex the solution to analyse
8. Add delicately **3 µl** of the solution to analyse on the lens without bubbles or spilling around the lens
9. Measure the concentration of your sample
10. Clean the lens with a tissue and sterile water
11. Repeat steps 6 to 10 three times per sample to analyse
12. Don't forget to clean the machine when finished!

Sample: pEX-S1 colony A1 concentration (ng/µl) : 519.4 ng/µl
Sample: pEX-S1 colony A2 concentration (ng/µl) : 475.6 ng/µl
Sample: pEX-S1 colony B1 concentration (ng/µl) : 521 ng/µl
Sample: pEX-S1 colony B2 concentration (ng/µl) : 413.6 ng/µl

20170928

Single Tube Transformation Protocol

Before You Start

Estimated bench time: 1 hour
Estimated total time: 2 hours (plus 14-18 hour incubation)
When transforming competent cells, both timing and temperature are very important. Use a lab timer, follow the incubation temperatures closely, and keep materials on ice when required.

- **Read through the entire protocol before starting!**

Materials

- DNA to be transformed :
- pSBIC3 dP E1_1 col 1 (ligation R/T 10 min RATIO 1:3) transform entire tube
- pSBIC3 dP E1_2 (ligation R/T 10 min RATIO 1:3) transform entire tube
- pSBIC3 dP E2 ((ligation R/T 10 min RATIO 1:3) transform entire tube
- Competent Cells (50 µl per sample) of DH5α
- 1.5 ml Microtubes
- SOC Media (950 µl per sample)
- Petri plates w/ LB agar and antibiotic **CAM** (2 per sample)

Operators : Ersin, Gaétan, Deshmukh

Equipment

- Floating Foam Tube Rack
- Ice & ice bucket
- Lab Timer
- 42°C water bath
- 37°C incubator
- Sterile spreader or glass beads
- Pipettes and Tips (10 µl, 20 µl, 200 µl recommended)
- Microcentrifuge

Method

1. **Thaw competent cells on ice:** This may take 10-15 min for a 260 µl stock. Dispose of unused competent cells. Do not refreeze unused thawed cells, as it will drastically reduce trans formation efficiency.
2. **Pipette 50 µl of competent cells into 1.5 ml tube:** 50 µl in a 1.5 ml tube per transformation. Tubes should be labeled, pre-chilled, and in a floating tube rack for support. Keep all tubes on ice. Don't forget a 1.5 ml tube for your control.
3. **Pipette 25 µl (entire tube) of DNA to transform (PSB1C3 dp : E1-1, E1-2 and E2) and add to a DH5α 1.5 ml tube:** Pipette from tube into appropriately labeled tube. Gently mix a few times. Keep all tubes on ice.
4. **Close 1.5 ml tubes, incubate on ice for 30 min:** Tubes may be gently agitated/flicked to mix solution, but return to ice immediately.
5. **Heat shock tubes at 42°C for 45 sec:** 1.5ml tubes should be in a floating foam tube rack. Place in water bath to ensure the bottoms of the tubes are submerged. Timing is critical.
6. **Incubate on ice for 5 min:** Return transformation tubes to ice bucket.
7. **Pipette 950 µl SOC media to each transformation:** SOC should be stored at 4°C, but can be warmed to room temperature before use. Check for contamination.

8. **Incubate at 37°C for 1 hours, shaking at 200-300 rpm**
9. **Pipette 200 µl of each transformation onto petri plates** Spread with sterilized spreader or glass beads immediately. This helps ensure that you will be able to pick out a single colony.
10. **Spin down cells at 6 800 x g for 3mins and discard 600 µl of the supernatant. Resuspend the cells in the remaining 200 µl, and pipette each transformation onto petri plates** Spread with sterilized spreader or glass beads immediately. This increases the chance of getting colonies from lower concentration DNA samples.
11. **Incubate transformations overnight (14-18 hr) at 37°C:** Incubate the plates upside down (agar side up). If incubated for too long, colonies may overgrow and the antibiotics may start to break down; un-transformed cells will begin to grow.

20170928

Single Tube Transformation Protocol

Before You Start

Estimated bench time: 1 hour
Estimated total time: 2 hours (plus 14-18 hour incubation)
When transforming competent cells, both timing and temperature are very important. Use a lab timer, follow the incubation temperatures closely, and keep materials on ice when required.

- **Read through the entire protocol before starting!**

Materials

- DNA to be transformed :
- pET32a dP E1_1 col 1 (ligation R/T 10 min RATIO 1:3) transform entire tube
- pET32a dP E1_2 (ligation R/T 10 min RATIO 1:3) transform entire tube
- pET32a dP E2 (ligation R/T 10 min RATIO 1:3) transform entire tube
- pET32.1a Eurofins tube (2 µl for transformation)
- Competent Cells (50 µl per sample) of DH5α
- 1.5 ml Microtubes
- SOC Media (950 µl per sample)
- Petri plates w/ LB agar and antibiotic **CARB** (2 per sample)

Operators : Ersin, Gaétan, Deshmukh

Equipment

- Floating Foam Tube Rack
- Ice & ice bucket
- Lab Timer
- 42°C water bath
- 37°C incubator
- Sterile spreader or glass beads
- Pipettes and Tips (10 µl, 20 µl, 200 µl recommended)
- Microcentrifuge

Method

12. **Thaw competent cells on ice:** This may take 10-15 min for a 260 µl stock. Dispose of unused competent cells. Do not refreeze unused thawed cells, as it will drastically reduce transformation efficiency.
13. **Pipette 50 µl of competent cells into 1.5 ml tube:** 50 µl in a 1.5 ml tube per transformation. Tubes should be labeled, pre-chilled, and in a floating tube rack for support. Keep all tubes on ice. Don't forget a 1.5 ml tube for your control.
14. **Pipette 20 µl (entire tube) of DNA to transform (pET32.1a dp : E1-1, E1-2 and E2) and add to a DH5α 1.5 ml tube:** Pipette from tube into appropriately labeled tube. Gently mix a few times. Keep all tubes on ice.
15. **Pipette 2 µl of DNA to transform pET32.1a eurofins tube and add to a DH5α 1.5 ml tube:** Pipette from tube into appropriately labeled tube. Gently mix a few times. Keep all tubes on ice.
16. **Close 1.5 ml tubes, incubate on ice for 30 min:** Tubes may be gently agitated/flicked to mix solution, but return to ice immediately.
17. **Heat shock tubes at 42°C for 45 sec:** 1.5 ml tubes should be in a floating foam tube rack. Place in water bath to ensure the bottoms of the tubes are submerged. Timing is critical.

18. **Incubate on ice for 5 min:** Return transformation tubes to ice bucket.
19. **Pipette 950 µl SOC media to each transformation:** SOC should be stored at 4°C, but can be warmed to room temperature before use. Check for contamination.
20. **Incubate at 37°C for 1 hours, shaking at 200-300 rpm**
21. **Pipette 200 µl of each transformation onto petri plates** Spread with sterilized spreader or glass beads immediately. This helps ensure that you will be able to pick out a single colony.
22. **Spin down cells at 6 800 x g for 3mins and discard 600 µl of the supernatant. Resuspend the cells in the remaining 200 µl, and pipette each transformation onto petri plates** Spread with sterilized spreader or glass beads immediately. This increases the chance of getting colonies from lower concentration DNA samples.
23. **Incubate transformations overnight (14-18 hr) at 37°C:** Incubate the plates upside down (agar side up). If incubated for too long, colonies may overgrow and the antibiotics may start to break down; un-transformed cells will begin to grow.